

IN THE CLAIMS:

Please cancel claims 107-125, 130-131, and 133-136, without prejudice or disclaimer.

Please add the following new claims:

B3  
8/2/01  
C1  
146. (New) A cytochrome P450 oxygenase variant comprising a mutation at a position corresponding to amino acid 331 of cytochrome P450<sub>cam</sub> from *P. putida* (SEQ ID NO:2).

147. (New) The cytochrome P450 oxygenase variant of claim 146, wherein the amino acid at the position corresponding to amino acid 331 is lysine.

148. (New) A cytochrome P450 oxygenase variant comprising at least one mutation at a position corresponding to amino acid 280 of cytochrome P450<sub>cam</sub> from *P. putida* (SEQ ID NO:2).

149. (New) The cytochrome P450 oxygenase variant of claim 148, wherein the amino acid at the position corresponding to amino acid 280 is leucine.

B3

150. (New) A cytochrome P450 oxygenase variant comprising at least one mutation at a position corresponding to amino acid 242 of cytochrome P450<sub>cam</sub> from *P. putida* (SEQ ID NO:2).

151. (New) The cytochrome P450 oxygenase variant of claim 148, wherein the amino acid at the position corresponding to amino acid 280 is phenylalanine.

152. (New) A cytochrome P450 oxygenase variant comprising at least one mutation at a position selected from the group consisting of amino acid positions 242, 280, and 331 of SEQ ID NO:2.

153. (New) The cytochrome P450 oxygenase variant of claim 152 comprising at least one mutation selected from the group consisting of:

- (a) lysine at position 331 of SEQ ID NO:2;
- (b) leucine at position 280 of SEQ ID NO:2; and
- (c) phenylalanine at position 242 of SEQ ID NO:2.

154. (New) A function-conservative variant of the variant cytochrome P450 oxygenase of claim 153.

B3

155. (New) An oxygenase enzyme variant encoded by a first polynucleotide that hybridizes to a second polynucleotide, which second polynucleotide encodes the cytochrome P450 oxygenase enzyme of claim 153.

156. (New) An evolved cytochrome P450 oxygenase variant having a catalytic activity at least two times the catalytic activity of wild-type cytochrome P450<sub>cam</sub> oxygenase from *P. putida* (SEQ ID NO:2) in promoting the oxygenation of an oxygenase substrate in the presence of an oxygen donor.

157. (New) An evolved cytochrome P450 oxygenase variant having a catalytic activity at least ten times the catalytic activity of wild-type cytochrome P450<sub>cam</sub> oxygenase from *P. putida* (SEQ ID NO:2) in promoting the oxygenation of an oxygenase substrate in the presence of an oxygen donor.

158. (New) An evolved cytochrome P450 oxygenase variant having a stability at least two times the stability of wild-type cytochrome P450<sub>cam</sub> oxygenase from *P. putida* (SEQ ID NO:2) in promoting the oxygenation of an oxygenase substrate in the presence of an oxygen donor.

159. (New) An evolved cytochrome P450 oxygenase variant having a stability at least ten times the stability of wild-type cytochrome P450<sub>cam</sub> oxygenase from *P.*

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B2

162. (New) The oxygenase variant of claim 161, wherein the detecting of the detectable composition comprises detection of at least one of ultraviolet light, color change, fluorescence, and luminescence.

Sub  
C3

163. (New) The oxygenase variant of claim 161, wherein

(a) the organic substrate is selected from the group consisting of naphthalene, 3-phenylpropionate, benzene, toluene, benzoic acid, anthracene, benzphetamine, and coumarin;

(b) the oxygen donor is selected from the group consisting of hydrogen peroxide and t-butyl peroxide; and

(c) the coupling enzyme is selected from the group consisting of horseradish peroxidase, cytochrome c peroxidase, tulip peroxidase, lignin peroxidase, carrot peroxidase, peanut peroxidase, soybean peroxidase, and peroxidase Novozyme® 502.

164. (New) An oxygenase variant evolved from a wild-type oxygenase enzyme, and having a stability at least ten times the stability of the wild-type oxygenase enzyme in promoting the oxygenation of an oxygenase substrate in the presence of an oxygen donor, which oxygenase variant was identified by a method comprising the steps of:

B3 (a) contacting a test enzyme variant with an oxygenase substrate and an oxygen donor under conditions allowing the formation of an oxygenated product if said test enzyme variant is an oxygenase enzyme; and

Sub C3 (b) providing a coupling enzyme which is capable of promoting the formation of a detectable composition from the oxygenated product; and

(c) detecting the detectable composition.

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165. (New) The oxygenase variant of claim 164, wherein the detecting of the detectable composition comprises detection of at least one of ultraviolet light, color change, fluorescence, and luminescence.

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Sub C4 166. (New) The oxygenase variant of claim 164, wherein

(a) the organic substrate is selected from the group consisting of naphthalene, 3-phenylpropionate, benzene, toluene, benzoic acid, anthracene, benzphetamine, and coumarin;

(b) the oxygen donor is selected from the group consisting of hydrogen peroxide and t-butyl peroxide; and

(c) the coupling enzyme is selected from the group consisting of horseradish peroxidase, cytochrome c peroxidase, tulip peroxidase, lignin peroxidase, carrot peroxidase, peanut peroxidase, soybean peroxidase, and peroxidase Novozyme® 502.

B3 167. (New) An cytochrome P450 oxygenase variant comprising a mutation at a position corresponding to at least one of amino acid 331, 280, and 242 of cytochrome P450<sub>cam</sub> from *P. putida* (SEQ ID NO:2), which cytochrome P450 oxygenase variant was identified by a method comprising the steps of:

- PC4
- (a) contacting a test cytochrome P450 oxygenase variant with an oxygenase substrate and an oxygen donor under conditions allowing the formation of an oxygenated product if said test enzyme variant is an oxygenase enzyme; and
  - (b) providing a coupling enzyme which is capable of promoting the formation of a detectable composition from the oxygenated product; and
  - (c) detecting the detectable composition.
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168. (New) The cytochrome P450 oxygenase variant of claim 167, wherein the detecting of the detectable composition comprises detection of at least one of ultraviolet light, color change, fluorescence, and luminescence.

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- PC5
169. (New) The cytochrome P450 oxygenase variant of claim 167, wherein
- (a) the organic substrate is selected from the group consisting of naphthalene, 3-phenylpropionate, benzene, toluene, benzoic acid, anthracene, benzphetamine, and coumarin;
  - (b) the oxygen donor is selected from the group consisting of hydrogen peroxide and t-butyl peroxide; and

B3

(c) the coupling enzyme is selected from the group consisting of horseradish peroxidase, cytochrome c peroxidase, tulip peroxidase, lignin peroxidase, carrot peroxidase, peanut peroxidase, soybean peroxidase, and peroxidase Novozyme® 502.

concluded

add c6